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Phytochemical Investigation and Anti-Inflammatory Properties of *Ludwigia adscendens* Extracts from Vietnam

Vu Hai Nam[1] , Nguyen Van Nhat Anh[2]

[1] Vietnam Academy of Science and Technology

Email: hainamvu2306@vast.com

[2] The Woodstock Academy, USA

Email: nguyennhatanh280808@gmail.com

Abstract: *Ludwigia adscendens (Onagraceae), also known as water primrose, is a common aquatic herb in Asia. It has long been used to treat inflammation, but its active components and anti-inflammatory properties have not been fully studied. This study extracted, isolated, and analyzed the major chemical components of L. adscendens extract to see how they affect inflammation. The material was extracted with ethanol, then the extract was separated into two parts: ethyl acetate and water. The ethyl acetate portion was then purified by column chromatography, and the structures of the isolated compounds were determined by MS and NMR spectroscopy. As a result, six compounds were isolated and identified: three triterpenoids (corosolic acid, uvaol, ursolic aldehyde), one flavonoid ((-)-epicatechin), and two phenolic acids (gallic acid, ferulic acid). Ethanol extracts, along with their ethyl acetate fractions and aqueous solutions, and isolated compounds, were evaluated for in vitro anti-inflammatory activity by measuring the inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. The ethyl acetate extract showed the strongest NO inhibition among the extracts, with an IC_{50} value of 32.09 µg/mL. Among the isolated compounds, ferulic acid, a phenolic acid, exhibited the most significant activity (IC_{50} = 20.66 µM), followed by gallic acid (IC_{50} = 23.52 µM), while the remaining isolates showed reduced activity. This study is a preliminary examination of the chemical composition of L. adscendens, thereby providing a scientific basis for the plant's anti-inflammatory potential. Therefore, the findings presented here open the door to further research focusing on optimizing extraction methods and evaluating in vivo anti-inflammatory activity.*

Keywords: Ludwigia adscendens; Anti-inflammatory; Nitric oxide; Triterpenoids; Phenolics; Ferulic acid.

1. Introduction

The genus *Ludwigia* L., belonging to the family Onagraceae, comprises approximately 75 species globally, predominantly distributed in tropical and subtropical regions. In Vietnam, six species have been recorded, among which *Ludwigia adscendens* (L.) Hara (syn. *Jussiaea repens* L.) is a prominent aquatic herb. Known locally as "Rau dua nuoc," this plant is characterized by its white pneumatophores and is widely distributed in wetlands, ponds, and rice paddies (Pham, 2000; Nguyen, 2003).

Ethnomedicinally, *L. adscendens* holds a significant position in the folk pharmacopeia of Vietnam, China, and India. It is traditionally utilized as a diuretic and for the treatment of diverse ailments including fever, cystitis, skin infections, mammary abscesses, parotitis, and dysentery (Do et al., 2006). Specific traditional remedies employ the dried plant in decoctions for treating glomerulonephritis, urinary tract infections, and digestive disorders (Vo, 2003). Despite these extensive traditional applications, scientific elucidation of the specific bioactive constituents responsible for these therapeutic effects, particularly regarding the Vietnamese population of this species, remains limited.

Previous phytochemical investigations on the *Ludwigia* genus have revealed the presence of flavonoids (e.g., quercetin, myricetin derivatives), phenolic acids (e.g., ellagic acid, gallic acid), and triterpenoids (e.g., oleanolic acid) (Barman et al., 2018; Mabou et al., 2015). Pharmacological studies have indicated that extracts from *Ludwigia* species possess antimicrobial, antioxidant, anticancer, and anti-inflammatory properties (Praneetha et al., 2018; Baky et al., 2021). However, the specific chemical profile of *L. adscendens* in Vietnam and the correlation between its isolated compounds and anti-inflammatory potency, specifically through the inhibition of inflammatory mediators like nitric oxide (NO), have not been systematically reported.

Inflammation is a complex biological response to harmful stimuli, and chronic inflammation is implicated in the pathogenesis of numerous diseases, including arthritis, cancer, and metabolic disorders. Macrophages play a central role in the inflammatory response by releasing pro-inflammatory mediators such as NO, cytokines, and prostaglandins upon stimulation by lipopolysaccharides (LPS) (Certad et al., 2017). Therefore, the inhibition of NO production in LPS-stimulated macrophages serves as a reliable model for screening potential anti-inflammatory agents.

This study aims to bridge the gap between traditional knowledge and modern phytochemistry by isolating the major secondary metabolites from the ethyl acetate fraction of *L. adscendens* aerial parts collected in Hai Duong province, Vietnam. Furthermore, we

evaluated the anti-inflammatory potential of the crude extracts and isolated pure compounds using the LPS-induced RAW 264.7 cell model, thereby identifying the key bioactive principles responsible for the plant's therapeutic properties.

2. Materials and Methods

2.1. Plant Material

The aerial parts of *Ludwigia adscendens* (L.) Hara were collected in Chi Linh city, Hai Duong province, Vietnam, in September 2023. The plant material was taxonomically identified, and a voucher specimen was deposited at the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam.

Figure 1. *Morphological characteristics of Ludwigia adscendens (L.) Hara (aerial parts). Source: internet.*

2.2. General Experimental Procedures

Chromatography: Thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 F254 plates (0.25 mm, Merck, Darmstadt, Germany). Column chromatography (CC) utilized silica gel 60 (0.040–0.063 mm, Merck), YMC RP-18 resins (30–50 µm, Fujisilica Chemical Ltd.), and Sephadex LH-20 (Merck).

Spectroscopy: Nuclear magnetic resonance spectroscopy (NMR): NMR measurements were performed on a Bruckker advance 500 MHz instrument with TMS as the internal standard, at the Institute of Chemistry - Vietnam Academy of Science and Technology. Using one-dimensional (^{1}HNMR, ^{13}C-NMR, and DEPT) and two-dimensional (HSQC, HMBC...) nuclear magnetic resonance spectroscopy techniques.

Bioassay Reagents: Lipopolysaccharide (LPS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Griess reagent, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM) and Fetal Bovine Serum (FBS) were obtained from Gibco (Grand Island, NY, USA). Cardamonin was used as the positive control.

2.3. Extraction and Isolation

Fresh aerial parts of *L. adscendens* (13 kg) were dried at 60^{0}C to constant weight, yielding 2 kg of dry material. The powdered material was extracted with ethanol (EtOH) under ultrasonication at room temperature (3 times). The solvent was removed under reduced pressure to yield the total ethanol extract (LA, 320 g). The LA extract was suspended in water and partitioned with ethyl acetate (EtOAc) to afford the ethyl acetate fraction (LAE, 140 g) and the water-soluble fraction (LAW, 179 g).

The LAE fraction (80 g) was subjected to silica gel column chromatography (CC) eluting with a gradient of Dichloromethane/Methanol (DCM/MeOH, 100:0 to 0:100 v/v) to yield six fractions (LAE1–LAE6).

- Fraction LAE1 (5.0 g) was chromatographed on silica gel using n-hexane/EtOAc (50:1-5:1). Sub-fraction LAE1.2 was recrystallized in acetone to afford compound 1 (8.8 mg). Sub-fraction LAE1.3 was further separated using n-Hexane/EtOAc (30:1 to 5:1) and recrystallized in acetone to afford compound 2 (10.2 mg).
- Fraction LAE2 (4.0 g) was re-chromatographed on silica gel with DCM/MeOH (99:1-20:1), followed by recrystallization in acetone to afford compound 3 (8.9 mg).
- Fraction LAE3 (6.5 g) was separated using Reverse Phase (RP-18) CC with MeOH/Water (3:7) to give three sub-fractions. Sub-fraction LAE3.2 was purified on Sephadex LH-20 (MeOH) to yield compound 4 (7.5 mg). Sub-fraction LAE3.1 was similarly purified on Sephadex LH-20 to yield compound 5 (7.2 mg).

- Fraction LAE4 (2.0 g) was subjected to RP-18 CC eluting with MeOH/Water (3:7). Sub-fraction LAE4.2 was recrystallized in methanol to yield compound 6 (7.5 mg).

2.4. Method for testing anti-inflammatory activity

Anti-inflammatory activity through inhibition of NO production in the RAW264.7 cell line in vitro was conducted at the Laboratory of Experimental Biology, Institute of Chemistry - Vietnam Academy of Science and Technology. RAW264.7 cells were cultured for 48 hours in DMEM medium at 37°C, 5% CO_2, and 10% FBS. The cell suspension was then transferred to 96-well plates at a density of 2.5 x 10^5 cells/well. The cells were stimulated with 2 µL LPS (0.1 mg/mL) for 24 hours and supplemented with different concentrations of reagent. Cardamonin was used as a positive control. The cell suspension was incubated with Griess reagent and $NaNO_2$ at different concentrations to construct a calibration curve. The reaction mixture was measured at λ = 570 nm. The percentage inhibition of NO production (%) was determined by the formula:

$$\%UC = \frac{[X_a]_{sample} - [X_a]_{LPS}}{[X_a]_{control} - [X_a]_{LPS}} \times 100$$

Where: $[X_a]$is the average NO concentration calculated based on the $NaNO_2$ standard curve.

The remaining cells after use for evaluating in vitro activities were supplemented with MTT solution (0.5 mg/mL in PBS), incubated for 4 hours in a 5% CO_2 incubator at 37°C. The crystalline metabolite formazan was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and its optical density was measured at λ = 540/720 nm on an Infinite F50 instrument (Tecan, Männedorf, Switzerland).

Cell survival rate (CS%) compared to control:

$$Cell\ inhibition\ rate\ (\%) = \left(\frac{OD_{sample}}{OD_{control\ (-)}}\right) \times 100 \pm \sigma$$

Standard deviation is calculated using the formula:

$$\sigma = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

The anti-inflammatory activity was evaluated by quantifying the accumulation of nitrite, a stable metabolite of NO, in the culture medium using the Griess reaction. RAW 264.7 cells were pre-treated with the test samples for 1 h, followed by stimulation with LPS (1 µg/mL) for 24 h. The culture supernatant (100 µL) was mixed with an equal volume of Griess reagent and incubated at room temperature for 10 min. Absorbance was measured at 570 nm. The nitrite concentration was calculated using a standard curve of sodium nitrite ($NaNO_2$). The percentage of inhibition was calculated as:

$$\%\ Inhibition = \frac{[NO]_{LPS} - [NO]_{sample}}{[NO]_{LPS} - [NO]_{control}} \times 100$$

Cardamonin was used as a positive control. IC_{50} values were determined using regression analysis.

3. Results and Discussion

3.1. Structure Elucidation of Isolated Compounds

Six compounds were isolated from the ethyl acetate fraction of *L. adscendens*. Their structures were elucidated based on spectroscopic analysis and comparison with literature data.

Compound 1: Corosolic acid

Obtained as a colorless powder, mp 250–252 °C. The ^{13}C-NMR spectrum exhibited 30 carbon signals, characteristic of a pentacyclic triterpene skeleton. Key signals included a carboxylic acid carbonyl at δ_C178.1 (C-28), two olefinic carbons at δ_C124.2 (C-12) and 138.3 (C-13), and two oxymethine carbons at δ_C82.3 (C-3) and 67.0 (C-2). The ^{1}H-NMR spectrum showed an olefinic proton at δ_H5.22 (d, J = 14.5Hz, H-12) and two oxymethine protons at δ_H3.41 (m, H-2) and 2.72 (d, J = 9.5Hz, H-3). The data were consistent with the structure of 2α, 3β-dihydroxy-urs-12-en-28-oic acid (corosolic acid) (Zong & Zhao, 2007).

Compound 2: (-)-Epicatechin

Obtained as a yellow powder, mp 239–241 °C. The ^{13}C-NMR spectrum displayed 15 carbon signals, including characteristic resonances for a flavonoid skeleton: δ_C82.9 (C-2), 68.1 (C-3), and 29.3 (C-4). The aromatic region showed signals corresponding to a catechol moiety (Ring B) and a resorcinol moiety (Ring A). The ^{1}H-NMR spectrum showed signals at δ_H4.18 (m, H-2) and 3.31 (m, H-3). The optical rotation $[\alpha]_D^{15} = -56.9$ indicated the (−)-isomer. The structure was identified as (−)-epicatechin (de Moura et al., 2022).

Compound 3: Uvaol

Obtained as a colorless powder. The ^{1}H-NMR spectrum revealed an olefinic proton at δ_H5.14 (t, J = 3.6Hz, H-12), an oxymethine proton at δ_H3.24 (d, J = 11.4Hz, H-3) indicative of a 3β-OH configuration, and seven methyl singlets. The ^{13}C-NMR spectrum showed 30 carbon signals, including an oxymethylene group at δ_C70.0 (C-28) and an oxymethine at δ_C79.1 (C-3). The data matched those of uvaol (urs-12-ene-3,28-diol) (Luna-Vazquez et al., 2016).

Compound 4: Ursolic aldehyde

Obtained as a colorless powder. The ^{13}C-NMR spectrum showed a characteristic aldehyde carbonyl signal at δ_C207.8 (C-28), distinguishing it from ursolic acid. Other signals included the olefinic carbons at δ_C126.3 and 137.8, and the oxymethine at δ_C79.1 (C-3). The ^{1}H-NMR spectrum confirmed the aldehyde proton at δ_H9.32. The structure was identified as 3β-hydroxy-urs-12-en-28-al (ursolic aldehyde) (Matebiel et al., 2019).

Compound 5: Gallic acid

Obtained as colorless needle crystals. The ^{1}H-NMR spectrum showed a singlet at δ_H7.20 (2H, s), corresponding to the equivalent H-2 and H-6 protons of a symmetric tetrasubstituted benzene ring. The ^{13}C-NMR spectrum displayed signals for a carboxyl group (δ_C170.6)and aromatic carbons oxygenated at positions 3, 4, and 5. The compound was identified as 3,4,5-trihydroxybenzoic acid (gallic acid) (Izu et al., 2024).

Compound 6: Ferulic acid

Obtained as a colorless powder. The ^{1}H-NMR spectrum exhibited an ABX aromatic system (δ_H7.06, 6.94, 7.11) and two trans-olefinic protons at δ_H7.73 and 6.31 (J = 16.2 Hz), characteristic of a cinnamic acid derivative. A methoxy group signal was observed at δ_H3.94. The structure was determined to be 3-methoxy-4-hydroxycinnamic acid (ferulic acid) (Takagaki & Nanjo, 2013).

3.2. In Vitro Anti-inflammatory Activity

The anti-inflammatory potential of the extracts and isolated compounds was assessed by measuring their ability to inhibit LPS-induced NO production in RAW 264.7 macrophages. The results are summarized in *Table 1*.

Table 1. *Inhibitory effects of extracts and compounds on NO production in LPS-stimulated RAW 264.7 cells.*

Sample	Highest tested concentration (µg/mL)	NO production inhibition (%)	IC_{50}
Negative control (-)	-	100,00 ± 2,8	-
Positive control (+) (Cardamonin)	81,32 ± 1,5	2,89 µM	
LPS	-	0,00 ± 2,0	-
LA	256	55,09 ± 2,1	55,88 µg/mL
LAE	256	61,57 ± 1,2	32,09 µg/mL
LAW	256	40,08 ± 2,2	-
1	256	42,43 ± 1,8	-
2	256	36,21 ± 1,3	-
3	256	50,85 ± 2,4	89,65 µM

4	256	39,65 ± 1,0	-
5	256	66,98 ± 1,5	23,52 µM
6	256	69,76 ± 2,6	20,66 µM

Note: Cells were treated with LPS (1 µg/mL) and samples for 24 h. Cardamonin was used as the positive control.

The ethyl acetate fraction (LAE) exhibited the strongest activity among the extracts with an $IC_{50}$of 32.09 µg/mL, suggesting that the bioactive constituents are concentrated in the semi-polar fraction. This aligns with the isolation of the active phenolics and triterpenoids from this fraction.

Among the isolated compounds, the simple phenolic acids demonstrated the most potent activity. Ferulic acid (6) was the most active compound with an $IC_{50}$of 20.66 µM, followed closely by gallic acid (5) with an $IC_{50}$of 23.52 µM. In contrast, the triterpenoids showed lower activity in this assay. Uvaol (3) exhibited moderate inhibition ($IC_{50} = 89.65$ µM), while corosolic acid (1) and ursolic aldehyde (4) showed less than 50% inhibition at the highest concentration tested (256 µg/mL).

The results showed that the extracts, ethyl acetate (LAE) and total ethanol (LA) extracts exhibited good in vitro anti-inflammatory activity with IC50 values of 32.09 and 55.88 µg/ml, respectively. Among the pure compounds, compounds 5 and 6 showed the best anti-inflammatory activity with IC50 values of 23.52 and 20.66 µM, respectively. The remaining extracts and pure compounds showed almost no activity at the highest tested concentration of 256 µg/ml.

3.3. Discussion

The genus Ludwigia has been used in folk medicine to treat inflammatory diseases. Recent studies have demonstrated the anti-inflammatory activity of this genus. Both L. octovalvis and L. perennis possess phenolic compounds, including flavonoids, tannins, and phenolic acid derivatives, which can suppress the production of TNF-α, IL-6, and IL-1β, and diminish NF-κB and MAPK activation, thus mitigating inflammation. Ethanol and aqueous extracts derived from L. octovalvis leaves exhibited considerable anti-inflammatory effects in a mouse model of carrageenan-induced paw edema. Furthermore, ethanol extracts of L. perennis significantly inhibited TNF-α and IL-6 in LPS-stimulated RAW 264.7 cells. Methanol extracts derived from the stems of *L. adscendens* demonstrated an ability to mitigate inflammation within a murine model of arthritis.

Although prior investigations concerning *L. adscendens* from diverse locales, including Bangladesh and Egypt, have identified saponins and flavonoids (Barman et al., 2018; Baky et al., 2022), the isolation of uvaol, corosolic acid, and ursolic aldehyde from the Vietnamese variant contributes novel phytochemical data to the existing body of knowledge. The observed anti-inflammatory effects reveal a noteworthy disparity among the various compound classes. Specifically, the phenolic compounds (5 and 6) exhibited a more pronounced inhibitory effect on NO production compared to the triterpenoids (1, 3, 4). Gallic acid (5) is recognized as a well-established anti-inflammatory compound. Prior investigations into the mechanisms of action have indicated that it suppresses nuclear factor kappa B (NF-κB) signaling, consequently diminishing the expression of pro-inflammatory cytokines, including TNF-α and IL-6 (Hsiang et al., 2013). The observed potency in our study (23.52 µM) aligns with existing literature, thereby validating its significance as a primary factor in the activity of the LAE fraction. Ferulic acid (6), identified as the most potent compound in our investigation (20.66 µM), is recognized for its anti-inflammatory properties, which are mediated through the inhibition of iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) expression (Zhang et al., 2018). Furthermore, its antioxidant capabilities contribute to the neutralization of reactive oxygen species (ROS), which are known to intensify inflammation. Conversely, the triterpenoids (1, 3, 4) exhibited comparatively reduced activity within this particular NO inhibition assay when contrasted with the phenolics. While corosolic acid (1) and its derivatives are typically recognized as strong anti-inflammatory agents (Baky et al., 2022), their efficacy may vary depending on the particular cellular model or biological pathway under investigation. As an illustration, corosolic acid is frequently cited for its anti-diabetic properties and its capacity to regulate metabolic processes (Zong & Zhao, 2007). Uvaol (3) exhibited moderate activity (89.65 µM), a finding consistent with research indicating its mechanism of action involves the inhibition of pro-inflammatory cytokines in colitis models; however, its direct inhibition of NO may be less effective than that of smaller phenolic compounds (Luna-Vazquez et al., 2016). The pronounced activity of the crude ethyl acetate extract (LAE, IC_{50} =32.09 µg/mL) relative to the pure triterpenoids implies a possible synergistic interaction among the extract's components, or that the extract's efficacy is primarily attributable to its high concentration of phenolic acids, including gallic and ferulic acid.

4. Conclusion

This study successfully investigated the chemical composition and anti-inflammatory potential of *Ludwigia adscendens* aerial parts from Vietnam. We isolated and characterized six compounds: corosolic acid, (-)-epicatechin, uvaol, ursolic aldehyde, gallic acid, and ferulic acid. Biological evaluation revealed that the ethyl acetate fraction possesses significant anti-inflammatory activity,

largely attributed to the presence of phenolic compounds like ferulic acid and gallic acid, which exhibited strong inhibition of NO production.

These findings support the traditional use of "Rau dua nuoc" in treating inflammatory conditions and suggest that the plant is a promising source of natural anti-inflammatory agents. Future research should focus on clarifying the molecular mechanisms of these compounds in *L. adscendens* and exploring the activity of the remaining fractions.

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